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Mosaicism in Pseudoachondroplasia

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Pseudoachondroplasia (PSACH) is a spondylo-epi-metaphyseal dysplasia characterized by disproportionate short stature, generalized ligamentous laxity, and precocious osteoarthritis. PSACH is caused by mutations in the cartilage oligomeric matrix protein (COMP) gene, which codes for a noncollagenous protein expressed in the territorial matrix of chondrocytes. Autosomal dominant inheritance has been demonstrated in many families; however, autosomal recessive inheritance has been suggested in some severe familial cases. Alternatively, germline/somatic mosaicism has been proposed and is credible, since it has been shown that dominantly inherited and sporadic cases of PSACH are caused by the same genetic defect. Here, we present evidence demonstrating somatic mosaicism in two PSACH families that were originally considered to represent autosomal recessive inheritance. The results of this study suggest that autosomal recessive inheritance is unlikely and all cases of PSACH should be studied for mutations in COMP. Am. J. Med. Genet. 70:287–291, 1997.

KEY WORDS: pseudoachondroplasia; spondylo-epi-metaphyseal dysplasia; skeletal dysplasia; somatic mosaicism; germline mosaicism; COMP; cartilage oligomeric matrix protein; mutation

INTRODUCTION

Pseudoachondroplasia (PSACH) is characterized by disproportionate short stature, generalized ligamentous laxity, and precocious osteoarthritis with normal face and intelligence. Radiologic findings include flat vertebrae, small irregular epiphyses, and flared metaphyses [Heselson et al., 1977; Wynne-Davies et al., 1986]. Recently it was demonstrated that PSACH is caused by mutations in the cartilage oligomeric matrix protein (COMP) gene [Briggs et al., 1995; Hecht et al., 1995]. COMP is a 524,000 Da protein expressed in the territorial matrix of chondrocytes, ligament, and tendon [Fife and Brandt, 1984; Hedbom et al., 1992; DisCesare et al., 1994; Hecht, 1996, unpublished results]. COMP is a member of the thrombospondin family of extracellular calcium-binding proteins and has greatest homology to thrombospondins-3 and -4. COMP is a homopentamer composed of an N-terminal domain required for pentamer formation, four EGF-like repeats, seven type three repeats (calcium-binding domains), and a C-terminal globular domain that may interact with other extracellular matrix proteins [Oldberg et al., 1992; Newton et al., 1994; Morgelin et al., 1992; Lawler, 1995]. Many of the identified PSACH mutations occur in the type three repeats of COMP, frequently exon 17B, suggesting that calcium-binding is important to the function of COMP and normal skeletal growth [Briggs et al., 1995; Hecht et al., 1995].

PSACH is generally inherited as an autosomal dominant disorder; however, severe familial cases with normal parents have been reported [Dennis and Renton, 1975; Heselson et al., 1977; Young and Moore, 1985; Wynne-Davies et al., 1986]. Germline mosaicism was proposed in the case of one family in which one of two affected sibs, born to normal parents, had an affected child [Hall et al., 1987]. Mosaicism may also be present in other familial PSACH cases with apparent autosomal recessive inheritance.

We describe somatic mosaicism for mutations in exon 17B of COMP in two families with recurrent PSACH in whom autosomal recessive inheritance was initially suggested [Hall et al., 1987].

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METHODS

Clinical Summary

Family 1 was described previously and the pedigree is shown in Figure 1a [Hall et al., 1987; Hecht et al., 1995]. Briefly, two sibs, individuals II-2 and II-3, were born to parents of average stature. Both affected individuals have physical findings typical of PSACH. The parents were phenotypically normal with the exception of the father, individual I-1, who was unable to extend his elbows, a characteristic finding in PSACH. Individuals I-1 and I-2 were 172.7 cm and 160.0 cm tall, respectively. Neither had any complaints or evidence of osteoarthritis. Individual I-2 died of a heart attack following breast cancer treatment at the age 65 years. Individual I-1, at age 78 years, is healthy. PSACH in this family was considered to have an autosomal recessive etiology until III-1 was diagnosed with PSACH at the age of 2 years [Hall et al., 1987].

The pedigree of family 2 is shown in Figure 1b. Two sibs, individuals II-1 and II-2, were diagnosed with typical PSACH between the ages of 2 and 3 years when linear growth decreased and a waddling gait was observed. The parents, I-1 and I-2, have average stature of 177.8 cm and 157.5 cm, respectively. They are phenotypically normal and do not have any limitation of joint movement or evidence of osteoarthritis.

Mutation Analysis

DNA extraction from lymphocytes, lymphoblasts, and individual hair root bulbs was performed using standard conditions [Sambrook et al., 1988].

PCR amplification of COMP exon 17B and single-strand conformational polymorphism (SSCP) analysis were performed as described previously [Hecht et al., 1995]. Three times the normal amount of DNA sample (30 μl) for individual I-1 was loaded on all gels. The overloaded sample was run in lanes separate from affected samples to avoid the possibility of contamination and repeated in multiple experiments. Additionally, the amount of DNA was titrated to establish how much DNA had to be loaded in order to see the mutant allele. In family 1, electrophoresis through 6% denaturing PAGE gels was performed at 55°C for 3 hours and silver stained according to Hecht et al. [1995]. In family 2, non-denaturing 4% PAGE gels were electrophoresed at 180 V for 40 minutes and visualized using ethidium bromide.

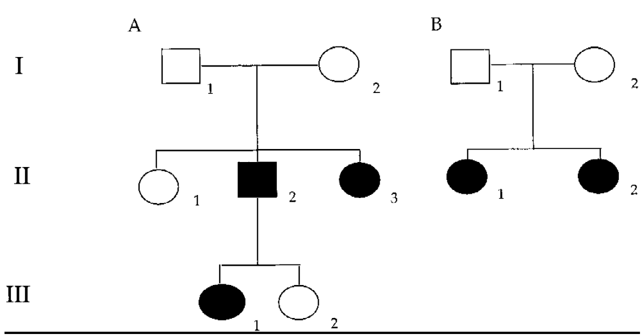

Fig. 1. Pedigrees of family 1 (**A**) and 2 (**B**).

Sequencing was performed using an ABI automated sequencer and results were compared to normal COMP sequence using GAP program in the GCG software package. Sequencing in family 2 was performed using L6R reverse primer for exon 17B [Hecht et al., 1995].

RESULTS

Family 1

SSCP analysis of exon 17B demonstrated two distinct banding patterns in all affected individuals. This PCR product was sequenced previously and a 3-base-pair deletion between nucleotides 1430 and 1455 was identified resulting in the loss of one of five aspartate residues [Hecht et al., 1995]. Previous linkage analysis showed that individual I-1 had passed his chromosome 19 marker alleles to all affected offspring [Hecht et al., 1993]. PCR amplification of exon 17B for all affected individuals demonstrated two fragments of equal intensity on silver stained denaturing polyacrylamide gels in contrast to all unaffected individuals who had only the upper band. Individual I-1 also had two bands in his overloaded DNA sample (3 times the normal amount, or 30 μl), with the mutant lower band having decreased intensity (Fig. 2). Individual I-1, family 1, demonstrated both SSCP banding patterns in his overloaded DNA sample with the normal upper bands having greater intensity than the mutant lower band (data not shown). PCR amplification of exon 17B from 50 hair root bulbs from I-1 demonstrated only the normal upper allele.

Family 2

Linkage analysis of polymorphic repeat markers from chromosome band 19p13 demonstrated that individuals II-1 and II-2 inherited the same chromosome 19 marker alleles from I-1 but received different alleles

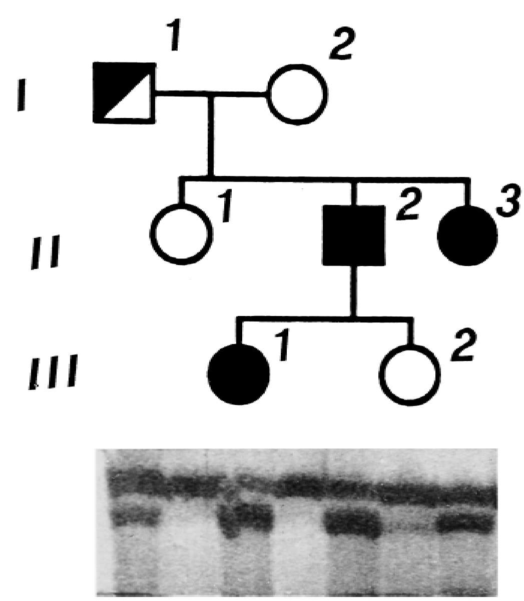

Fig. 2. PCR product demonstrating lower allele associated with the 3 bp deletion in all affected individuals. Sample from mosaic parent was overloaded and demonstrated a lighter lower band.

from individual I-2 [Hecht, 1996, unpublished data]. PCR products of exon 17B run on 4% PAGE gels disclosed a distinctive variant for individuals I-1, II-1, and II-2 (data not shown). Sequencing revealed a 3-base-pair deletion of nucleotides between 1367 and 1372, which results in a loss of a glutamic acid residue (Fig. 3). This region contains an AGGAGG repeat, so we are unable to determine the exact residues that are deleted. This sequence change was present in both PSACH sibs (II-1 and II-2), the unaffected, mosaic mother (I-2) but not the unaffected father (I-1).

DISCUSSION

We have demonstrated that two families presenting with recurrent PSACH in sibs is a result of somatic and germline mosaicism in a parent. Figure 4 demonstrates the exon 17B proposed calcium binding domain with the E-F hand configuration [Hecht et al., 1995]. Two distinct 3-base-pair deletions in exon 17B of COMP have been identified and both are predicted to disrupt a calcium-binding domain of COMP. The mutations in COMP exon 17B reported here segregate with the disease phenotype, with the exception of the unaffected parents who are mosaics for the mutation. We were able to detect mosaicism in lymphocyte DNA by overloading the DNA samples from individual I-1 in family 1. The hair root DNA analysis from this individual did not reveal the mutation, suggesting that this mutation is absent in, or present in only a very small proportion of this tissue. A 3-base pair deletion removing a conserved glutamic acid residue was identified in the second family.

Germline mosaicism for a dominant mutation can appear as autosomal recessive inheritance when ≤ 2 affected children are born to apparently normal parents. In the case of germline mosaicism, phenotypically normal individuals may transmit several gametes that are clonal descendants of a single progenitor cell in which a de novo mutation occurred during the early development of the parent [Hall, 1988]. The manifestation of such a mutation in the mosaic parent may range from none or minimal to a severe generalized effect or a segmental or patchy effect. Individual I-1 in family 1 is most likely manifesting a patchy distribution, since he has limited elbow extension but no other signs of PSACH. This physical finding suggests that the cartilage and/or tendon and/or ligament tissues in this region may be expressing the mutation as COMP is made in all three of these tissues (Hecht, 1996, unpublished data). In contrast, individual I-1 in family 2 demonstrated none of the phenotypic traits of PSACH.

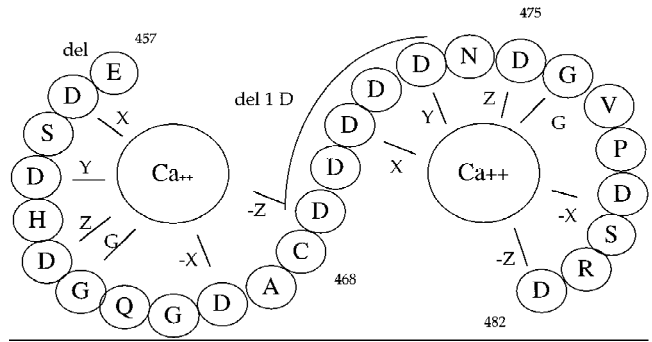

Fig. 4. Calcium-binding domain of exon 17B of COMP with nucleotide deletions indicated. The configuration of the domain is based on studies in the thrombospondin gene family.

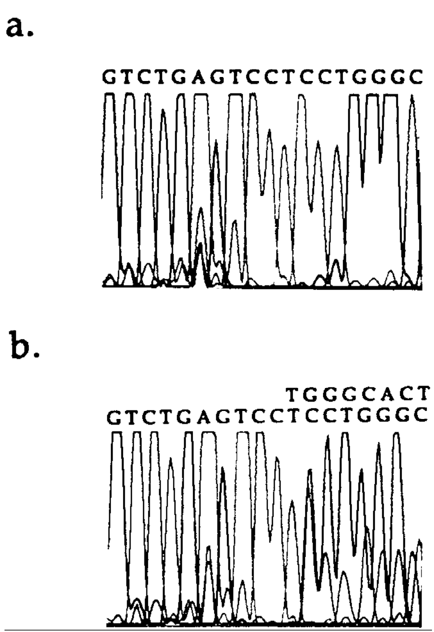

Fig. 3. Sequencing results from family 2 with (**a**) normal parent and (**b**) PSACH child. The 3 bp deletion disrupts the normal reading frame but the normal sequence can be observed along with the mutant sequence.

The phenotypic effect of mosaicism presumably depends on the nature of the mutation and the cells in which it is expressed. It is possible that a spontaneous mutation that occurs early in development, and involves germline and somatic cells, may be selected against in some somatic cells that require the gene product for normal development, while the mutation is tolerated in the germ cells [Hall, 1988]. For example, Fryns and Van Den Berghe [1986] reported an asymmetrical skeletal dysplasia affecting the entire right side of the body which closely resembled PSACH.

Germline/somatic mosaicism has been documented in two other autosomal dominant skeletal dysplasias, achondroplasia (ACH) and osteogenesis imperfecta (OI) type II. Evidence of germline mosaicism in ACH was based upon the recurrence of the disease in sibs born to normal parents and subsequent dominant inheritance of ACH in succeeding generations [Fryns et al., 1983; Reiser et al., 1984; Dodinval and LeMarec, 1987; Bowen, 1974]. Subsequently, Stoilov et al. [1995] demonstrated germline/somatic mosaicism in the fibroblast growth factor receptor 3 (FGFR3) gene in the achondroplasia family reported previously by Fryns et al. [1983]. Somatic mosaicism has been the mechanism suggested to explain ACH in an individual with typical involvement of his entire skeleton except for the second to fifth fingers of his left hand. The presence of normal and proportionate fingers were attributed to a back mutation [Rimoin and McKusick, 1969].

Molecular evidence for germline/somatic mosaicism

was reported in perinatal lethal type II OI [Cohn et al., 1990]. Two infants affected with OI type II, born to the same father but different mothers, were shown to have the same single nucleotide mutation in the COL1A1 gene. The same mutation was detected in somatic DNA from the father's hair root bulbs, lymphocytes, and sperm, but not in dermal fibroblast DNA. The presence of somatic/germline mosaicism suggests that the mutation occurred prior to segregation of the germline and somatic cell progenitors. Although the father has no clinical signs of OI, tissue analysis demonstrated the mutation in some of his somatic tissues. In a second report, a father with a mild form of OI was mosaic for the same mutation that caused perinatal lethal OI in his son. About half of the father's COL1A1 alleles in fibroblasts demonstrated the mutation, but a lower proportion of lymphocyte and sperm cells were found to have the mutation. His milder phenotype reflects the mosaic distribution of the mutant allele in his tissues. This mutation must have occurred during the father's embryonic development, prior to segregation of fibroblast, germline and hematopoietic lineages [Wallis et al., 1990].

Mosaicism poses a genetic counseling dilemma. The recurrence risk for a new dominant mutation is very small; however, because some families have germline/somatic mosaicism, a 1% recurrence risk is quoted. The exact risk to subsequent offspring would depend on the proportion of germ cells carrying the mutation which is determined by the time at which the mutation occurred during parental germline differentiation and would differ for each family. Based on statistical inference, the recurrence risk for a dominant disorder would be less than 5% if only one sib was affected and 20–35% if $\geqslant 2$ sibs were affected [Bakker et al., 1989]. Empiric recurrence risks ranging from 4 to 6% for sporadic ACH and OI type II have been proposed based on observed recurrences in families presenting with germline mosaicism [Hall et al., 1987; Cohn et al., 1990]. However, the true risk for any individual family depends on the proportion of germ cells affected in the carrier parent. Since the molecular abnormalities are known for PSACH, ACH, and OI type II, it is possible to analyze the parents for somatic/germline mosaicism by examining different tissues in order to determine a more accurate genetic risk. Lymphocytes may be a particularly useful tissue because blood precursor stem cells and germline cells both derive from the same source, e.g., yolk sack. Thus, germline mosaicism may be accurately reflected in lymphocytes as long as there is no selective disadvantage in this tissue. Prenatal diagnosis is now available for these conditions.

An autosomal recessive form of PSACH has yet to be identified. There are no pathognomonic or diagnostic manifestations that separate the reported autosomal dominant and autosomal recessive cases of PSACH. Furthermore, mutations in COMP cause both dominantly inherited and sporadic PSACH [Briggs et al., 1995; Hecht et al., 1995]. We propose that cases of "autosomal recessive" PSACH are examples of germline/somatic mosaicism that should be evaluated for COMP mutations.

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